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SPERMATOLOGICAL EVIDENCE SUPPORTS THE TAXONOMIC PLACEMENT OF THE AUSTRALIAN ENDEMIC HAIRY STONE CRAB, *LOMIS HIRTA* (DECAPODA: ANOMURA: LOMIDAE)

Christopher C. Tudge

Zoology Department, University of Queensland, Brisbane, Qld 4072, Australia
Present address: Crustacea, Museum of Victoria, 71 Victoria Crescent, Abbotsford, Vic. 3067, Australia

Abstract

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An ultrastructural investigation of the spermatozoal morphology of the Australian endemic hairy stone crab, *Lomis hirta*, has revealed a unique sperm morphology supporting placement within its own family and superfamily. The spermatophore and spermatozoal morphology agree with morphological evidence that place this crab within the infraorder Anomura. the relatively unmodified spermatozoon suggests a basal origin for this taxon (with respect to other anomurans) and may be similar to the ancestral sperm type for many families in this infraorder. An independent lineage from the base of the Anomura has been previously suggested for *Lomis* on the basis of many morphological synapomorphies being shared with representatives from disparate families of the Anomura.

Introduction

Previous classifications of the decapod infraorder Anomura (Borradaile, 1907; Glaessner, 1969), recognised the four superfamilies, Thalassinoidea, Paguroidea, Galatheoidea and Hippoidea. More recently the thalassinoids have been excluded from the Anomura and the constituent anomuran superfamilies redefined as the Paguroidea, Lomoidea, Galatheoidea and Hippoidea (McLaughlin, 1983b; McLaughlin and Holthuis, 1985; Poore, 1994). McLaughlin and Holthuis stated that the term 'Anomala' was the more appropriate name for these constituent superfamilies (minus the thalassinoids), but the term 'Anomura' is more commonly used and has been adopted herein.

The superfamily Lomoidea contains the monospecific genus *Lomis* in the family Lomidae. This hairy stone crab is endemic to the rocky southern coasts of Australia and like its porcellanid relatives is very crab-like in appearance. *Lomis hirta* was first described as a hairy porcellanid by Lamarck (1818), then Bouvier (1894, 1895) considered it a symmetrical hermit crab, but this enigmatic crab has since been elevated to its own family and superfamily (McLaughlin, 1983a). Pilgrim (1965) reassessed the literature and examined fresh specimens and came to the conclusion that *Lomis* was a basal offshoot of the paguroids (along with the Pylochelidae) and that this basal position was indicative of its sharing many characters with the paguroids, galatheoids and thalassinoids. McLaughlin (1983a) similarly placed *Lomis* in a basal position, with respect to the Anomura, but had it evolving independently as a distinct evolutionary line from a common ancestral stock. *Lomis* continues to be problematic in regard to its relationship to the remainder of the Anomura (McLaughlin, 1983b; Martin and Abele, 1986; Richter and Scholtz, 1994).

The use of spermatozoal morphology for systematic, taxonomic and phylogenetic studies, initially pioneered by workers such as Koltzoff (1906) and Retzius (1909), has been successfully applied to the Crustacea (Jamieson, 1991) and in the Anomura and Brachyura in particular (Tudge, 1992, 1995a, b; Jamieson, 1994, 1995; and references therein). In this paper, the ultrastructure of the spermatozoa of *Lomis hirta* is described for the first time and the phylogenetic implications are discussed.

Methods

The single male specimen of *Lomis hirta* (Lamarck, 1818) was collected and fixed by Dr Gary Poore, Curator of Crustacea, Museum of Victoria, Australia from Flinders Reef, Victoria in September 1993. The male reproductive material (both testes including the ducts of the vasa deferentia) was removed from the crab and immediately fixed in cold glutaraldehyde for a

minimum of 2 hours at 4°C then posted to Brisbane at ambient temperature where the remainder of the fixation and embedding process was carried out.

Light microscopy

For light microscopy, glutaraldehyde-fixed sperm were viewed and photographed under an Olympus BH2 Nomarski interference contrast microscope. Micrographs were taken with an attached Olympus OM-2 camera.

Transmission electron microscopy

The standard fixation procedure (outlined below) for transmission electron microscopy was carried out in a Lynx -el. Microscopy Tissue Processor (Australian Biomedical Corporation, Ltd, Mount Waverley, Victoria, Australia), after the initial glutaraldehyde fixation and first phosphate buffer wash.

Portions of the testis (approximately 1 mm^3) were fixed in 3% glutaraldehyde in 0.2 *M* phosphate buffer (pH 7.2), with 3% sucrose added, for a minimum of 1 h at 4°C. They were washed in phosphate buffer (3 washes in 15 min), post-fixed in phosphate buffered 1% osmium tetroxide for 80 min; similarly washed in buffer and dehydrated through ascending concentrations of ethanol (40–100%). After being infiltrated and embedded in Spurr's epoxy resin (Spurr, 1969), thin sections (500–800 Å thick) were cut on a LKB 2128 UM IV microtome with a diamond knife. Sections were placed on carbon-stabilised colloidin-coated 200 μm mesh copper grids and stained (according to Daddow, 1986) in Reynold's lead citrate (Reynolds, 1963) for 30 s, rinsed in distilled water, then 6% aqueous uranyl acetate for 1 min, Reynold's lead citrate again for 30 s and a final rinse in distilled water. Micrographs were taken on an Hitachi H-300 transmission electron microscope at 80 kV.

Results

Spermatophore morphology

The spermatophores of *Lomis hirta* were only viewed in thick sections for transmission electron microscopy and the dimensions and morphology can only be estimated. The spermatophores are pedunculate, having the sperm-filled ampulla attached to a long thin stalk which in turn is connected to a basal plate or pedestal. The entire spermatophores have been estimated at approximately 300 μm in length and the ampullar dimensions at 100 μm long × 50 μm wide (Fig. 1A). The spermatophore wall (Fig. 1E), which constitutes the ampullar region of the spermatophore, is shown at the electron microscope level to be composed of a single homogeneously granular electron-dense layer (Fig. 2A) and to be of variable thickness.

Spermatozoal morphology

At the light microscope level the spermatozoa of *Lomis hirta* are irregular in outline, but basically globular in form with one to three small vertices which may be extended into microtubular arms (Figs 1B–E). Up to two microtubular arms have been observed in a single spermatozoon but sometimes three vertices are apparent (Fig. 1B). A ring-shaped acrosome vesicle is obvious on one surface of the sperm cell. The entire sperm cell can be up to 6μm in diameter (refer to Figs 2C and 4 throughout). In all figures the acrosomal or apical end of the spermatozoon is considered the anterior pole while the opposite, basal or nuclear end is posterior.

The acrosome vesicle is an irregular, inverted cup-shaped structure, approximately 2.5 μm wide, and is broadly penetrated posteriorly by the perforatorial chamber (Figs 2B, C; 3A, B, D). The acrosome vesicle wall is relatively thin and is composed of three distinctive zones. The most external region of the vesicle is made up of an interrupted layer of extremely electron-dense material embedded in a granular zone of lesser electron density. The extremely electron-dense material is interpreted to be the equivalent of the dense operculum, which normally caps the acrosome vesicle in anomurans, but its extension around the entire acrosome vesicle is unusual (Figs 2B, C; 3A, B, D, F). As mentioned this opercular layer is not a continuous, evenly distributed structure but forms an interrupted dense layer. The electron-dense, granular zone, here interpreted as the outer acrosome zone, surrounds the operculum and composes the majority of the thin acrosome vesicle. Occurring between this combined dense outer acrosome layer and the perforatorial chamber wall is a zone of similar granularity, the inner acrosome zone (Figs 2C; 3A, B, D-F). This latter zone is more electron-pale than the outer acrosome zone.

The perforatorial chamber invaginates into the acrosome vesicle from the posterior end and extends almost to the anterior most tip. It has a broad posterior region which tapers anteriorly to a blunt point (Figs 2C; 3A). The open, posterior end is slightly constricted when compared to the greatest width of the perforatorial chamber. The perforatorial chamber contents can be divided into two distinct regions. Internally there is a

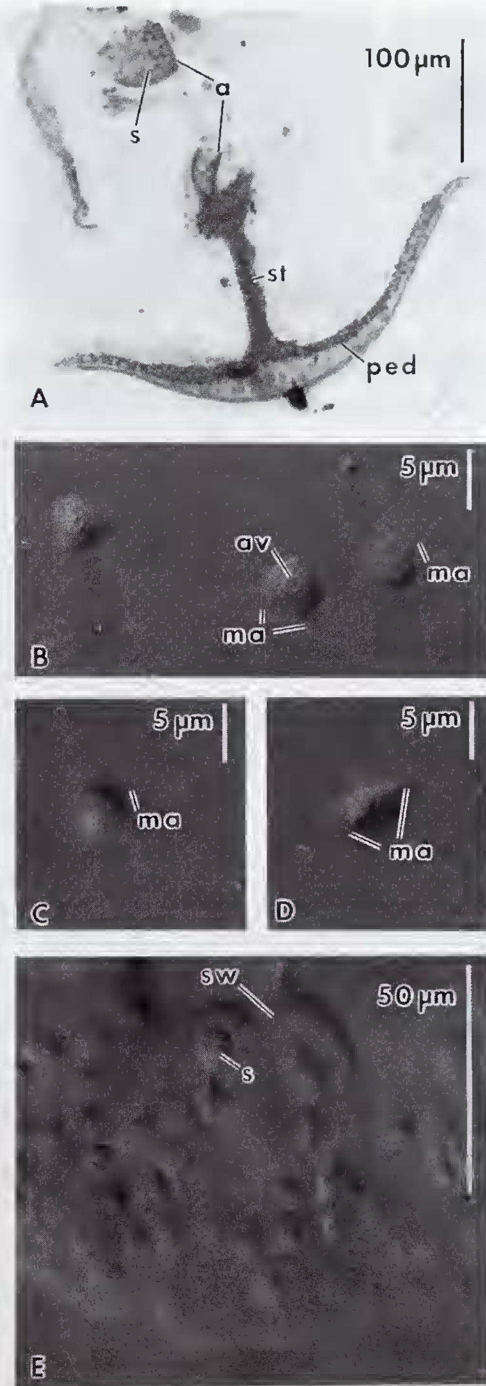

Figure 1. A–E *Lomis hirta* (Lomidae). A. Toluidine stained thick resin section showing partially sectioned spermatophore and contained spermatozoa. B–D. Light micrographs of spermatozoa showing one or two microtubular arms. E. Light micrograph of spermatozoa extruded from the vas deferens. Note: the thick spermatophore wall. Scale bars as indicated (original).
a = ampulla; av = acrosome vesicle; ma = microtubular arm; ped = pedestal; s = spermatozoon; st = stalk; sw = spermatophore wall.

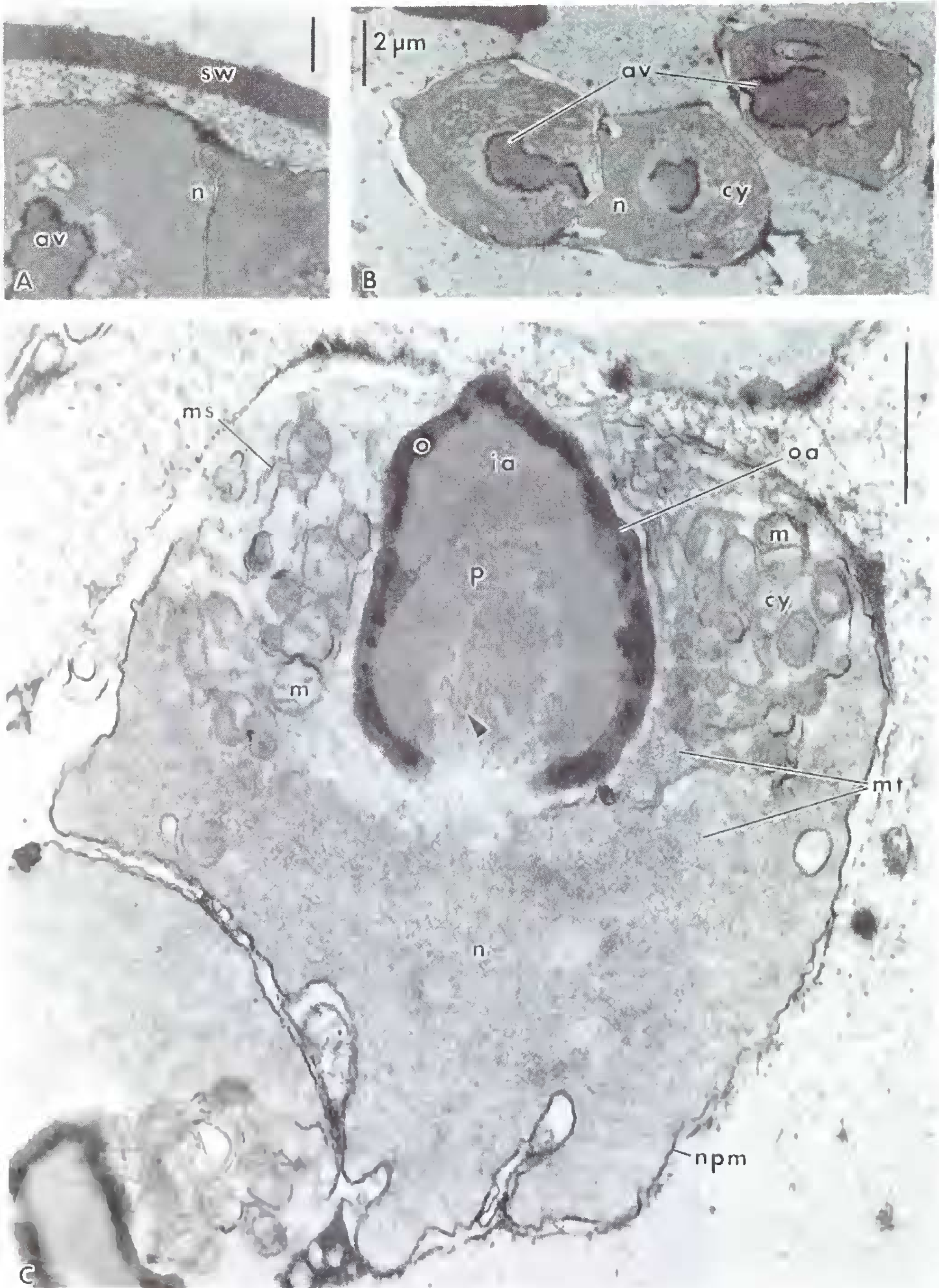

Figure 2. A–C *Lomis hirta* (Lomidae). Transmission electron micrographs of spermatozoa. A. Detail of spermatophore wall surrounding spermatozoa. B. Low power shot of three spermatozoa. C. Longitudinal section (LS) of a spermatozoon. Arrowhead indicates probable actin filaments. Scale bar = 1µm, except where indicated (original).
av = acrosome vesicle; cy = cytoplasm; ia = inner acrosome zone; m = mitochondrion; ms = membrane system; mt = microtubules; n = nucleus; npm = nucleo-plasma membrane; o = operculum; oa = outer acrosome zone; p = perforatorial chamber; sw = spermatophore wall.

core of longitudinally arranged striations, similar in appearance to actin filaments, while external to this is a homogeneous, finely granular region of moderate electron density (Figs 2C; 3A, B; 4). The posterior contents of the perforatorial chamber appear continuous with the cytoplasm beneath the acrosome vesicle.

The cytoplasm is very extensive and completely surrounds the acrosome vesicle, with the widest areas lateral to it (Figs 2C; 3D, F; 4). Anterior and posterior to the acrosome vesicle the cytoplasm is reduced to a thin layer with no obvious organelles present, except for a pair of centrioles which occur beneath the perforatorial chamber (Fig. 3C). Lateral to the acrosome vesicle the cytoplasm contains many organelles including an anterior region of loosely aggregated membranes and associated with these, many small spherical mitochondria, of which some appear cristate and others more degenerate (acristate) (Figs 2C; 3D, F). Scattered amongst these organelles are short and long bundles of microtubules which represent internal sections of the microtubular arms. Some bundles of microtubules are seen to extend into the nuclear material (Figs 3D, E).

The nucleus of the spermatozoa of *Lomis hirta* is irregular in form but retains a basically globular appearance (Figs 1B-D). It is composed of homogeneous, coarsely granular material of moderate electron density and is surrounded externally by a thickened nucleo-plasma membrane (Figs 2B, C; 3D; 4). No distinct membrane separates the cytoplasm from the nucleus. As previously mentioned small sections of the internal microtubular bundles are apparent in the nucleus (Fig. 2C).

Discussion

The paucity of information concerning the morphology of the spermatophores of *Lomis hirta* make it difficult to do more than speculate about their structure. It can be ascertained that the spermatophores are pedunculate (Fig. 1), and therefore typical of the Anomura studied to date (Tudge, 1991, 1995a). Until further morphological and ultrastructural studies are carried out on the spermatophore of this crab it will not be known if it has a spermatophore morphology that substantiates its morphological and spermatozoal uniqueness. The homogeneous, granular appearance of the spermatophore wall in *Lomis* (Fig. 2A) is similar to that recorded in the spermatophores of several pagurid hermit crabs, the parapagurid, *Sympagurus*, the galatheid, *Allogalathea*, the hippid, *Hippa*, and several porcellanid species (Tudge, 1995a).

The spermatozoon of *Lomis hirta* (Figs 2, 3, 4) possesses spermatozoal characters, such as microtubular arms (possible three?) and a concentrically arranged acrosome vesicle posteriorly penetrated by a perforatorial chamber, which justify its position in the Anomura but its spermatozoal morphology is distinct enough to warrant its own family and superfamily. Some of the characteristics of the spermatozoon of *Lomis* are considered unique. The variation in sperm cell shape shown within an individual is unusual and the amorphous form of the spermatozoa (Figs 1B-E; 2B), and to a lesser extent, the acrosome vesicle, distinguishes *Lomis* from other investigated anomurans (Hinsch, 1980, 1991; Tudge, 1992, 1995a, b). The small acrosome vesicle completely embedded in the cytoplasm has not been recorded for any other anomuran spermatozoon and is only approximated by that seen in the thalassinoids, *Axius* and *Callianassa* (Tudge, 1995a, b). The microtubular bundles, which appear to be concentrated in the cytoplasm, can also extend into the nucleus (Figs 2C; 3D-F). It is not known if these microtubular bundles, which are the bases of the microtubular arms, are cytoplasmic in origin (as in other investigated anomurans) or nuclear in origin (as seen in the spermatozoa of astacideans, palinurans and some thalassinoids and brachyurans). Although the sperm cells appear to have three vertices, only one or two microtubular arms have been seen on a single spermatozoon (Figs 1B-D) and it is not known if three microtubular arms is standard for *Lomis*. Three microtubular arms have been recorded for all investigated paguroids and galatheoids, with the exception of the Porcellanidae; which, with the hippids and thalassinoids, have four or more microtubular arms (Tudge, 1995a). Another unique feature of the spermatozoa of *Lomis* is the discontinuous electron-dense operculum, which surrounds the entire acrosome vesicle (Figs 2C; 2A, B, D-F) and not just the apical pole, as in all other investigated anomurans. An alternative theory concerning this structure is that it is not an operculum at all, but simply another dense zone of the acrosome vesicle. This possibility assumes that the operculum is therefore lacking which would similarly be an autapomorphy.

The Lomidae have been assigned to a basal position in the Anomura, based on adult somatic characters (Pilgrim, 1965; McLaughlin, 1983a, b; Martin and Abele, 1986; Richter and Scholtz,

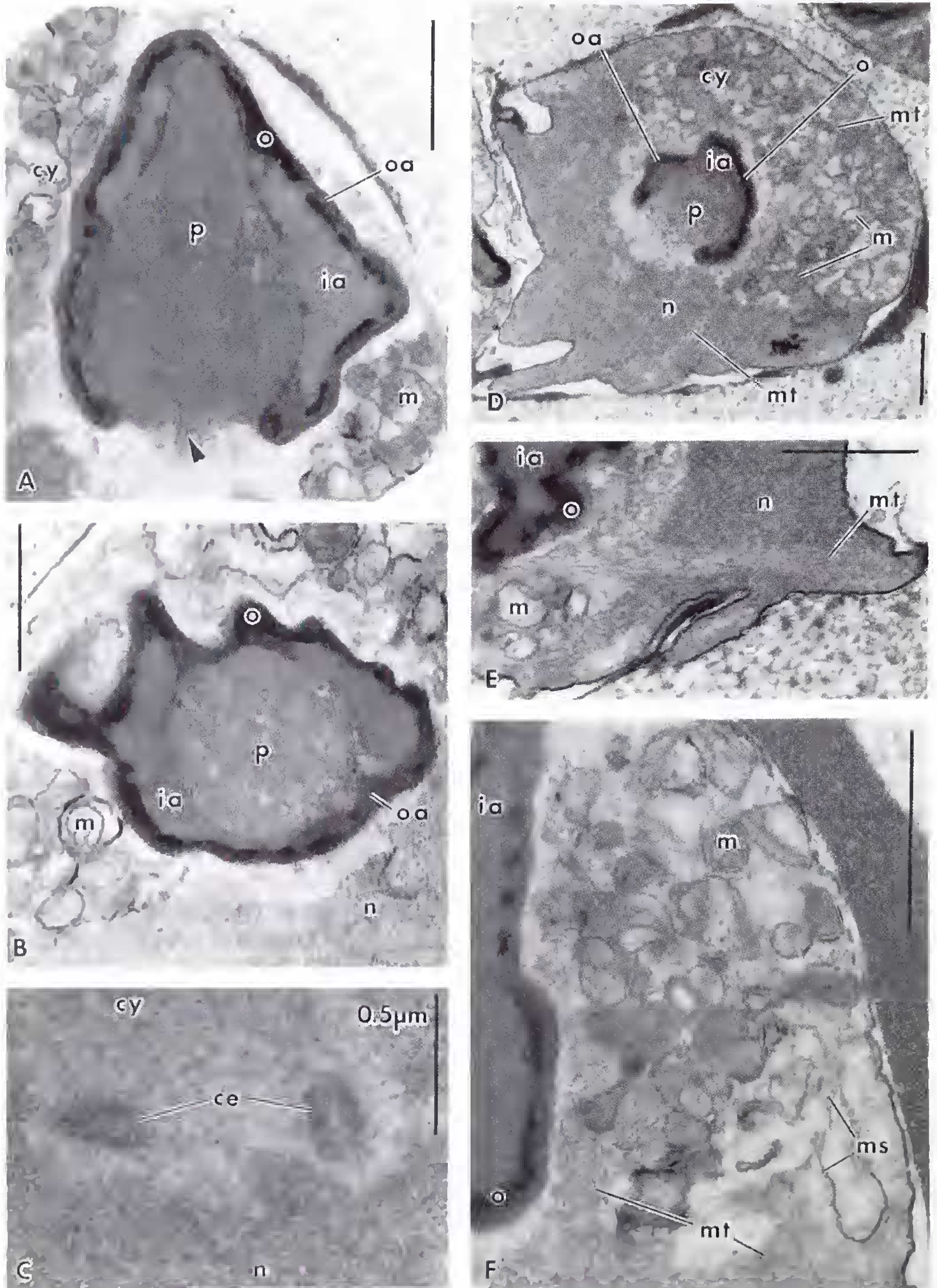

Figure 3. A–F. *Lomis hirta* (Lomidae). Transmission electron micrographs of spermatozoa. A. Detail of longitudinal section (LS) of acrosome vesicle. Arrowhead indicates probable actin filaments. B. Transverse section (TS) through acrosome vesicle. C. Detail of pair of centrioles. D. Oblique section through a spermatozoon showing long internal bundle of microtubules. E. Detail of microtubular bundle. F. Detail of cytoplasmic organelles. Scale bars = 1 µm, except where indicated (original).

ce = centriole; cy = cytoplasm; ia = inner acrosome zone; m = mitochondrion; ms = membrane system; mt = microtubules; n = nucleus; o = operculum; oa = outer acrosome zone; p = perforatorial chamber.

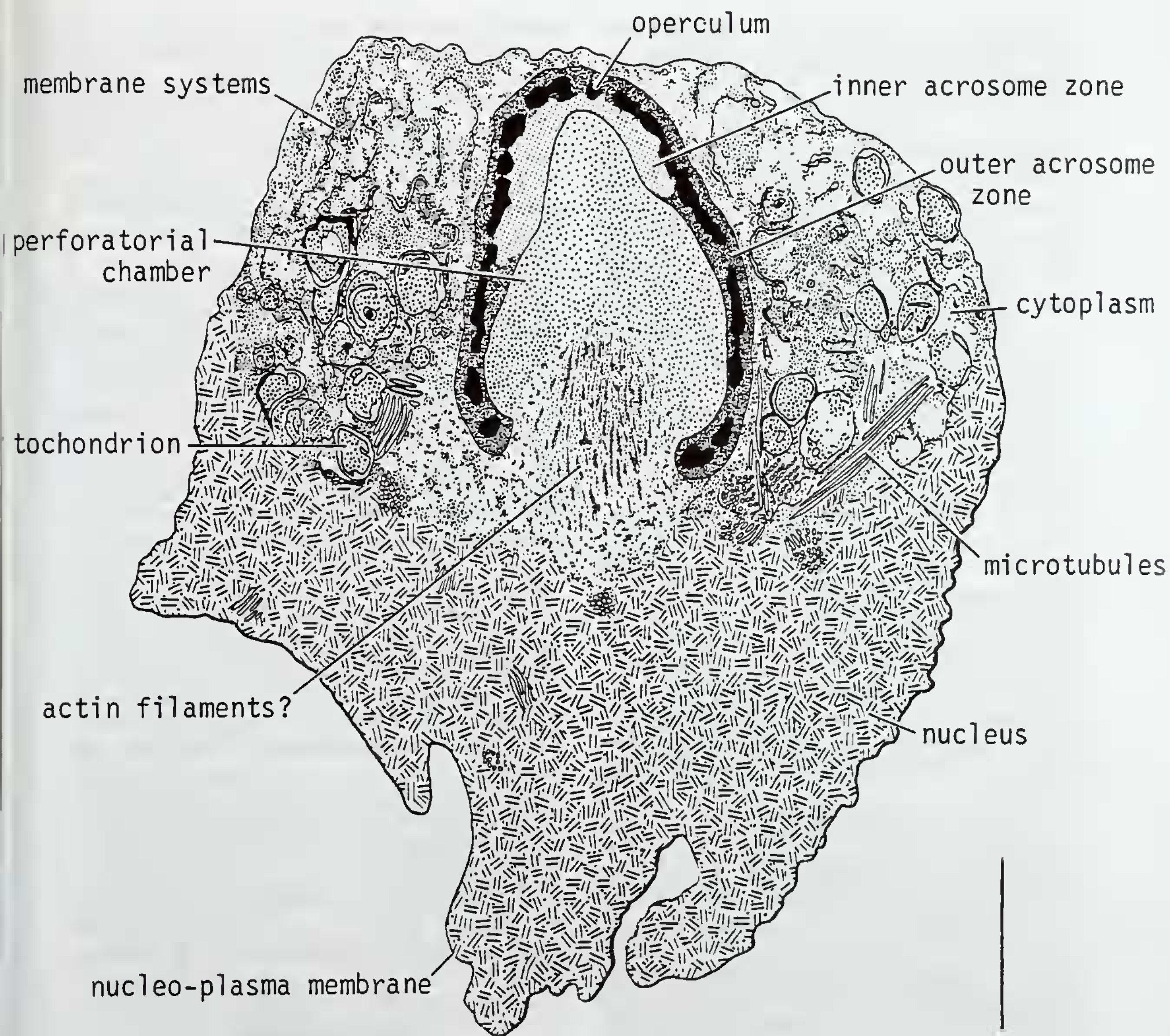

Figure 4. *Lomis hirta* (Lomidae). Semidiagrammatic longitudinal section of a spermatozoon, based on a tracing of a micrograph. Scale bar = 1μm (original).

1994), but there are differences between their conclusions. Not all authors dealt with all anomuran taxa. Pilgrim (1965) thought the family to be an early offshoot of the Anomura (within which he included the Thalassinidea). McLaughlin (1983a: 435) stated "the Lomidae represents a distinct evolutionary lineage within the Anomala, sharing the basic characters of the infraorder, but having evolved independently from the ancestral stock common to all of its major taxa", and decided it was a specialised and highly evolved taxon within the infraorder. Martin and Abele (1986) thought the Lomidae to be the sister family to the Lithodidae, together sister taxa to the Paguroidea. Richter and Scholtz (1994) placed the Lomidae as the sister group of the Paguroidea (including Lithodidae in the latter superfamily) based on uropod morphology.

The fact that the phylogenetic relationship of *Lomis* has sparked such debate and still remains unclear is reflected in its lack of recognisable morphological apomorphies and its sharing of numerous synapomorphic characters with more remotely related anomuran taxa. Like many other families in the Anomura (the Hippidae, Lithodidae, Pylochelidae and Aeglidae, for example) the Lomidae is only represented as fossils from the Recent (Glaessner, 1969) and therefore palaeontological evidence of their evolution is limited.

The spermatozoal data supports the views presented by Pilgrim (1965) and McLaughlin (1983a) that *Lomis* is basal to, or a separate lineage from, the ancestor of the Anomura + Thalassinidea (Pilgrim, 1965) or Anomala (anomurans excluding thalassinideans) (McLaughlin, 1983a). This same spermatozoal data is less supportive of the view expressed by Richter and Scholtz (1994) that the Lomidae is the sister taxon of the Paguroidea and does not support the close relationship between *Lomis* and lithodids suggested by Martin and Abele (1986). Although the spermatozoal morphology in the Lithodidae is only described from light microscope drawings of *Lithodes maja* (see Retzius, 1909), it can be clearly ascertained that the overall spermatozoal morphology is unlike that of *Lomis* and more similar to investigated paguroids (Tudge, 1992, 1995a, b). A close link between *Lomis* and lithodids seems unlikely on the present spermatological evidence. Poore (1994) clearly showed a sister group relationship between the Thalassinidea and the Anomura based on morphological characters but the position of *Lomis* in this analysis is not known as it was not one of the anomuran taxa used. The spermatozoa of *Lomis* appear to share several characters in common with two previously investigated thalassinoids (*Axius* and *Callianassa*) but a detailed comparison of spermatozoal morphology between the Lomoidea and Thalassinoidea will have to wait pending a more thorough investigation into the spermatozoal form and diversity within the latter superfamily.

The ancestor of some or all of the anomuran families may have had a spermatozoal morphology very similar to that of *Lomis* and the diversity (admittedly restricted by a conservative ground-plan) of sperm morphology encountered in extant families of the Anomura could have emerged through modification of this ancestral sperm form. It is conceivable that the anomuran spermatozoal type (in the Paguroidea in particular) developed from a lomid-like morphology by 1) increasing the size and complexity of the acrosome vesicle, 2) restricting the operculum to the apical pole, 3) shifting the acrosome vesicle to a superior position on the cytoplasm and 4) establishing three, equidistant microtubular arms. The lomid lineage may have independently evolved from this ancestor but retained the spermatozoal morphology which was ancestral to the remainder of the Anomura. The lack of distinct morphological apomorphies, the sharing of morphological characters with as many as nine other anomuran families and the newly presented spermatological evidence, indicating a unique and particularly unmodified sperm morphology, would appear to vindicate a basal or ancestral position of *Lomis* in relation to the remainder of the Anomura.

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